



Discussions for a

National Molecular Microscopy Laboratory

Sriram Subramaniam, Ph.D.

Laboratory of Cell Biology
Center for Cancer Research
National Cancer Institute

September 2014



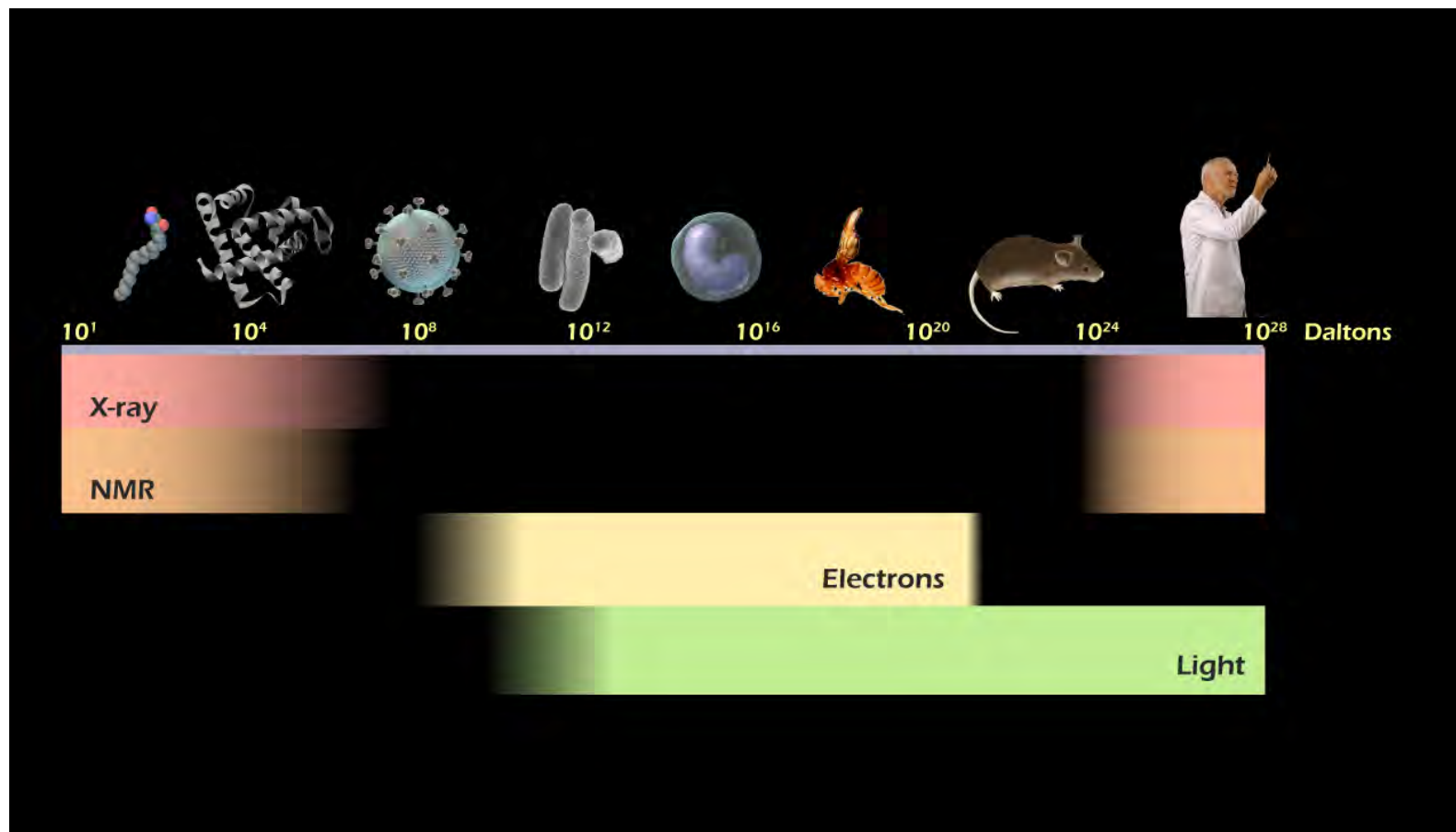


Outline of presentation

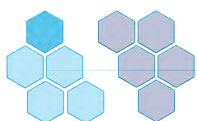




Imaging gaps in biology and medicine



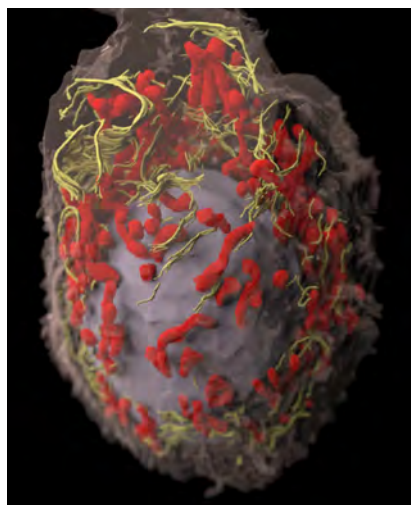
Subramaniam, *Curr. Opin. Microbiol.* (2005)





2005-2014: A 10-year plan in molecular microscopy

3D mapping of
cancer cells



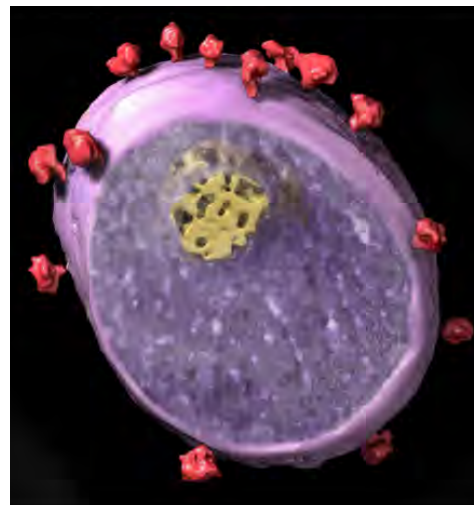
~ 15000 nm

Spatial architecture of
signal transduction



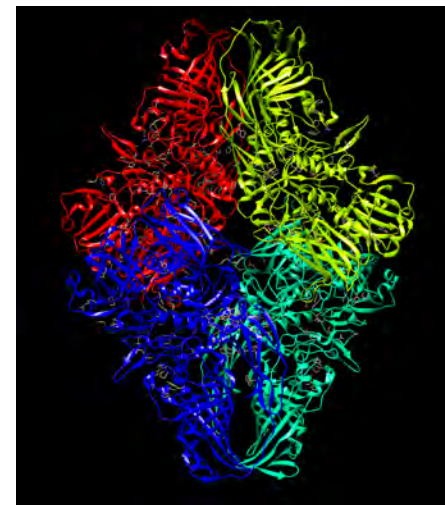
~ 1500 nm

Mechanisms of HIV
entry

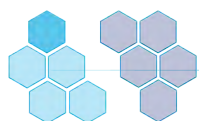


~ 150 nm

Protein complexes
in metabolism

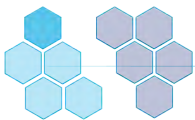
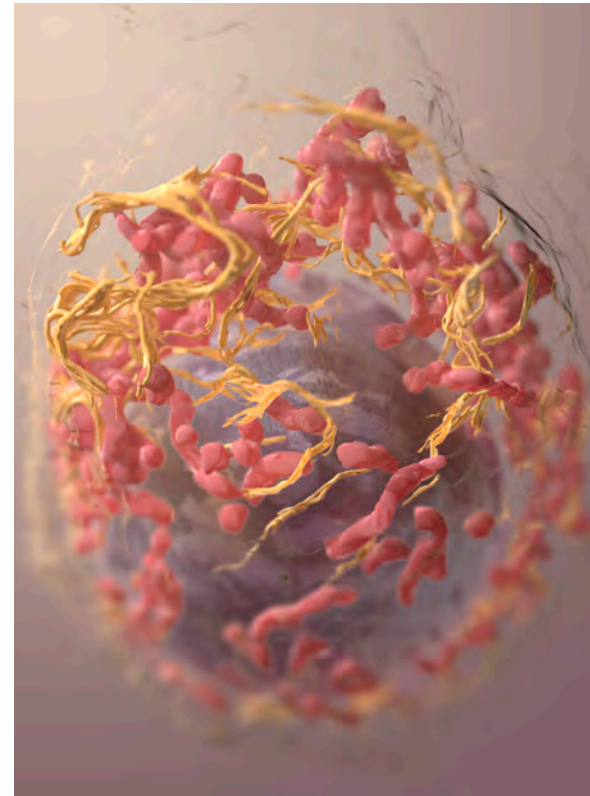
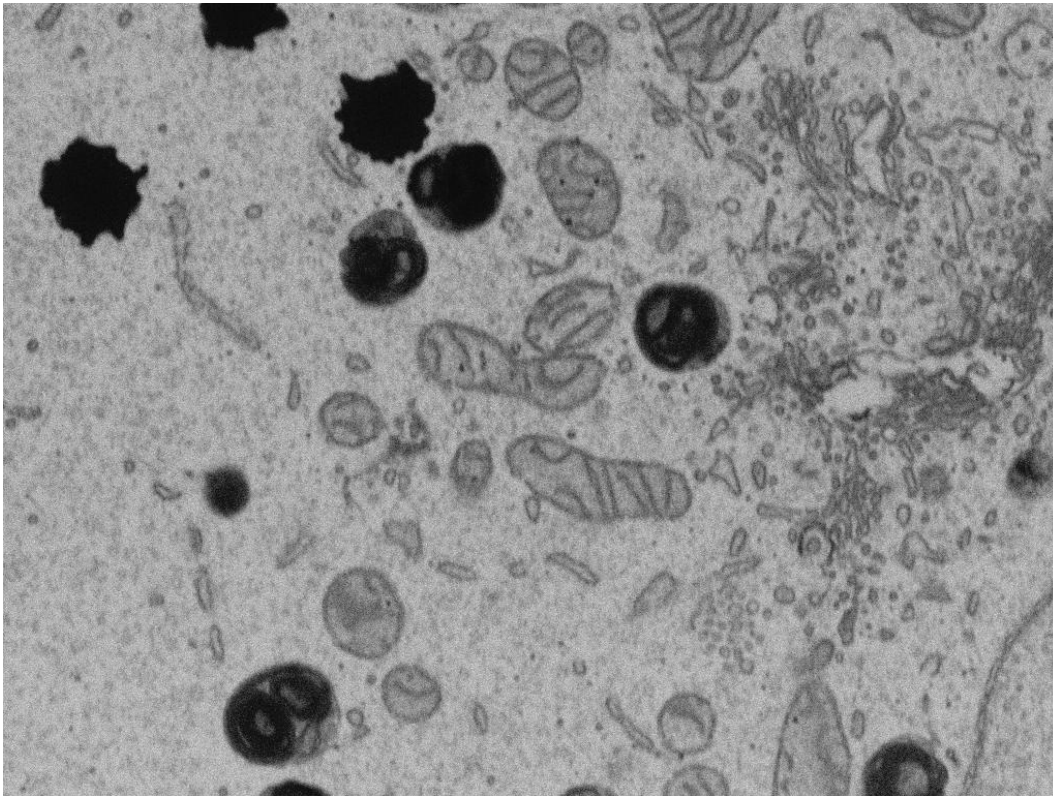


~ 15 nm



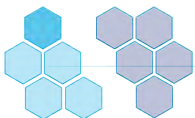
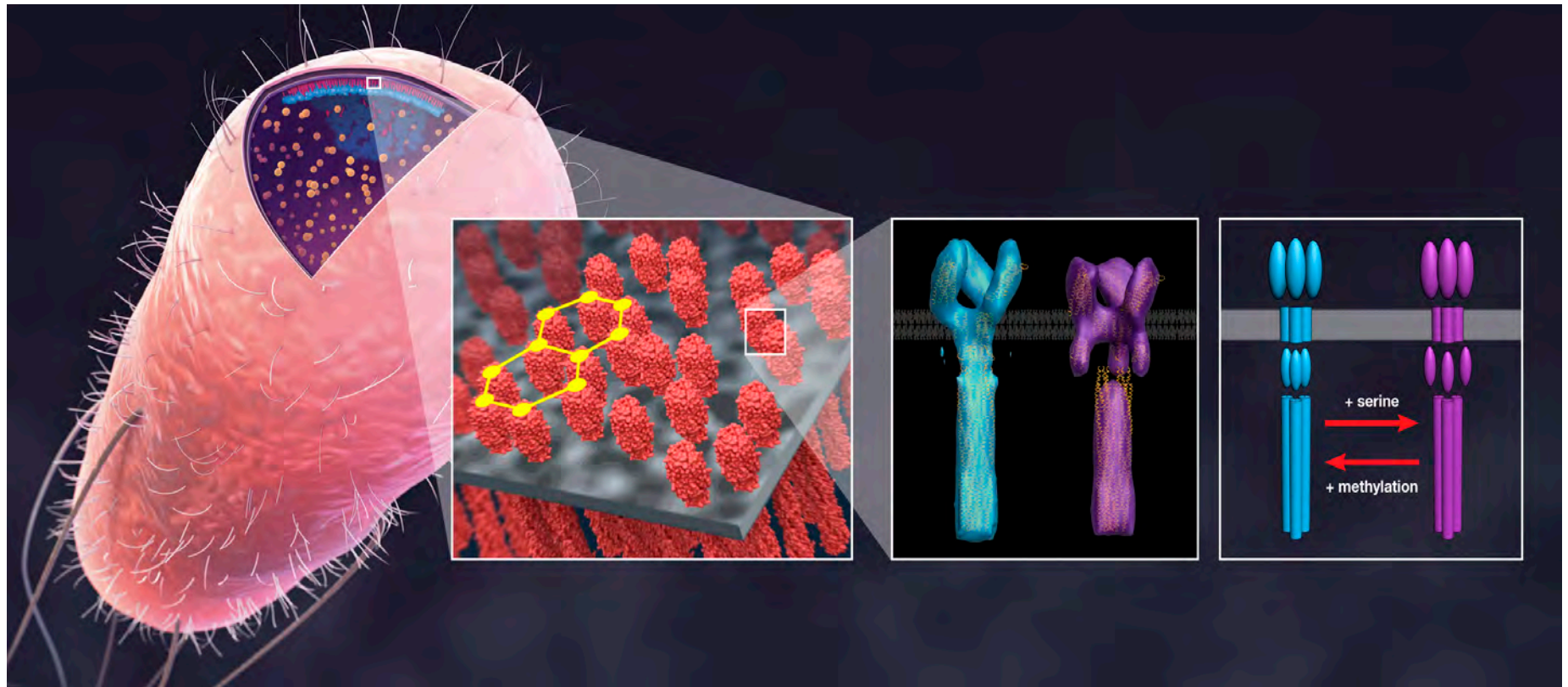


Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue



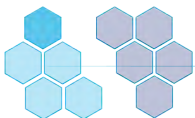
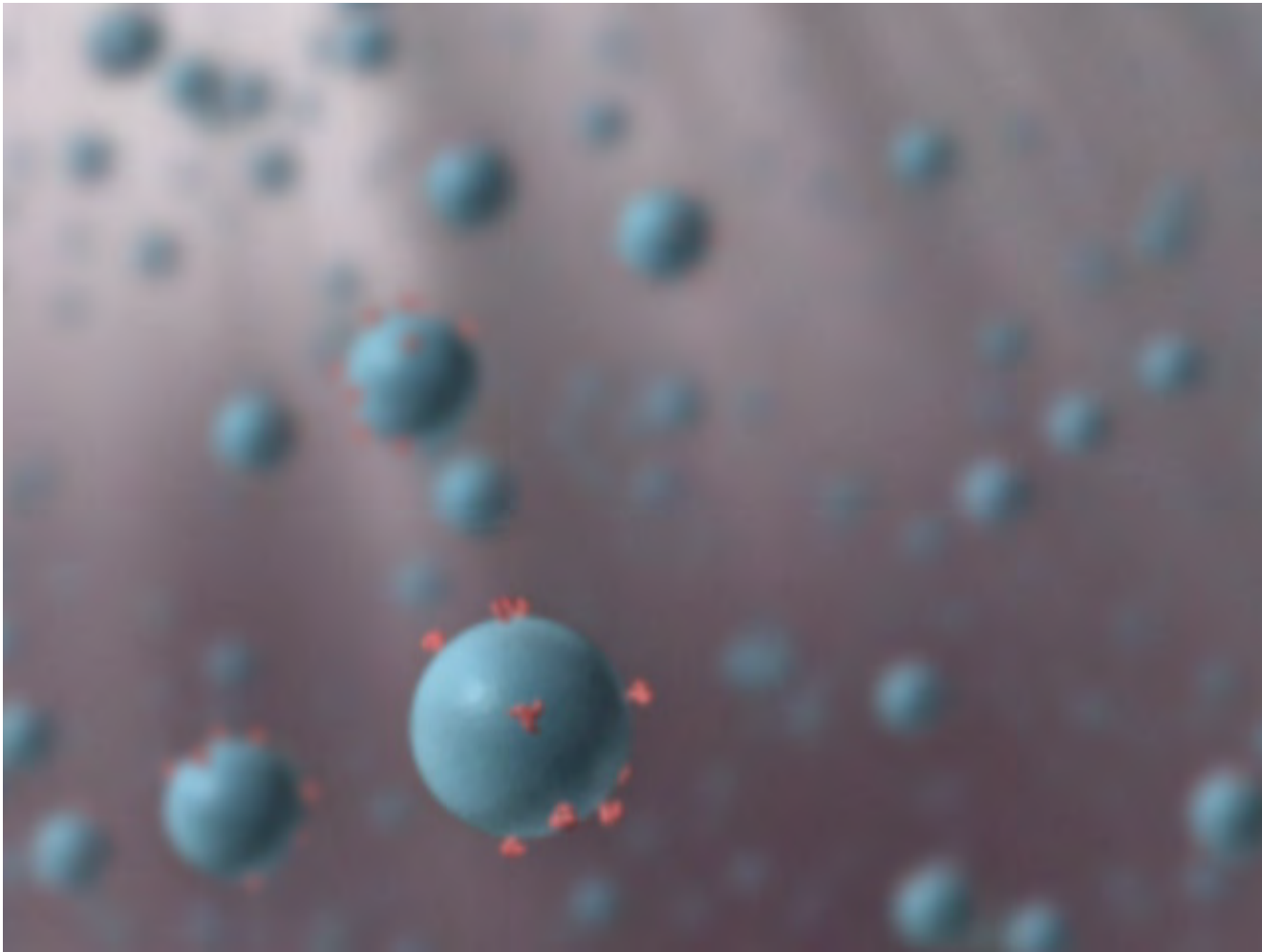


Imaging receptor arrays and signaling complexes in intact cells



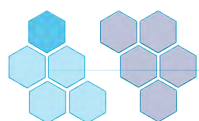
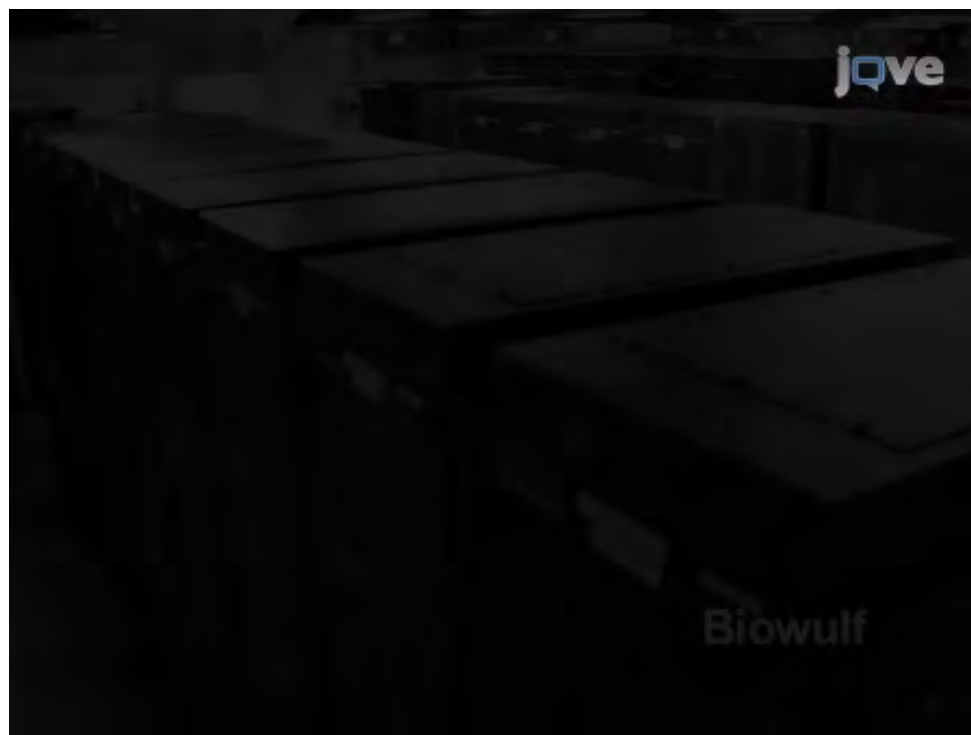


Cryo-electron tomography of HIV



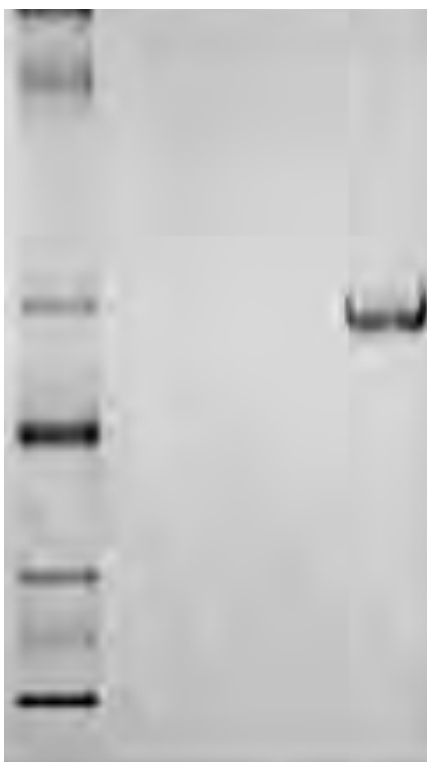


From spikes to structure





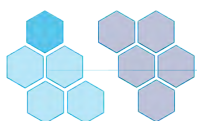
Structure determination without crystallography: A biochemist's dream



Purified protein complex



Vision for a structure determination machine

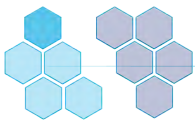
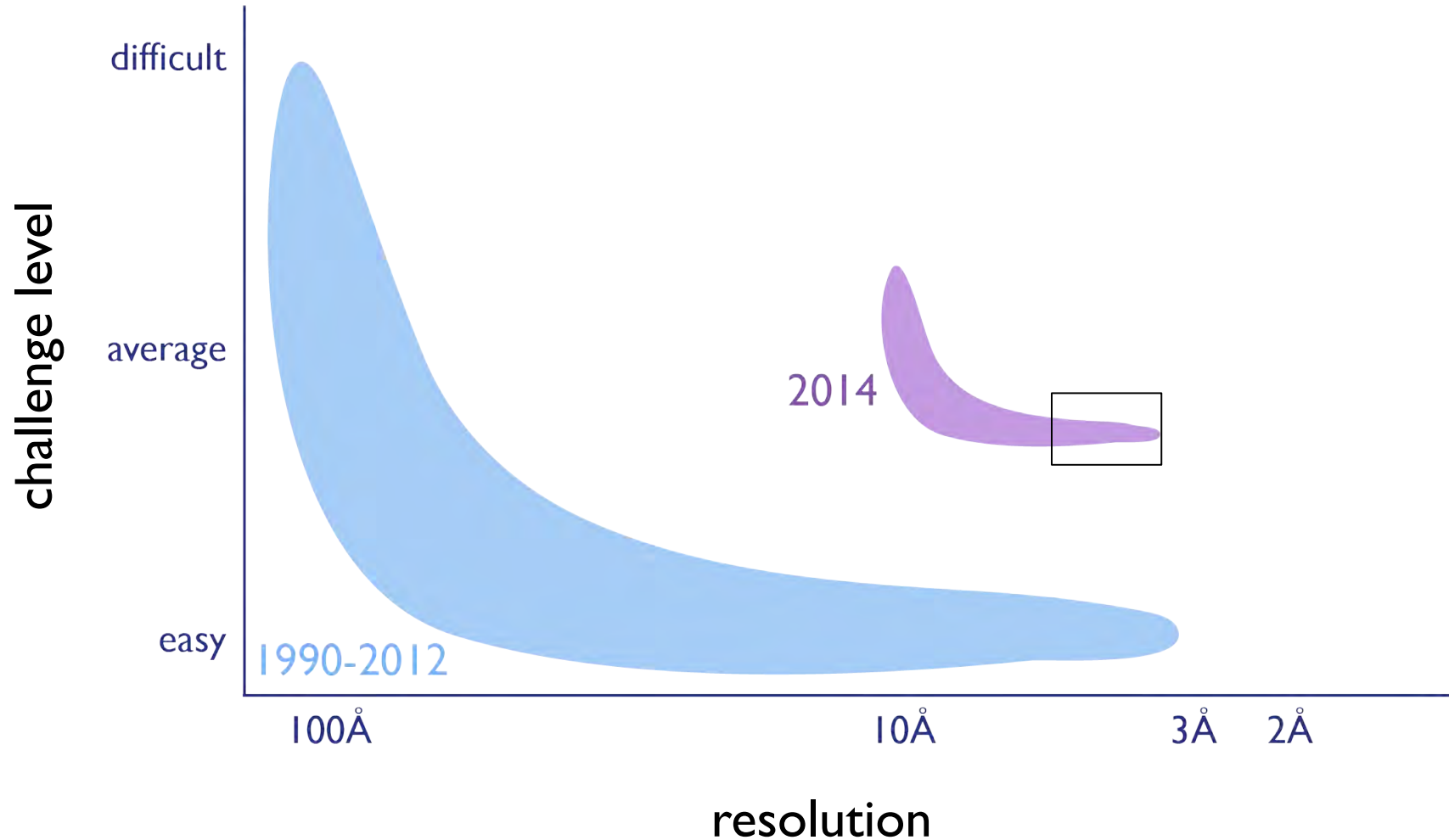


S. Subramaniam, U.S. Patent No. 6,987,266 B2 (issued Jan 2006)





Recent progress in cryo-EM field





The emergence of atomic resolution cryo-EM

Henderson, Quart. Rev. Biophys. (1995)

The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules

RICHARD HENDERSON

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

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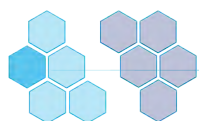
1990: First atomic resolution model from electron crystallography of 2D protein crystals (3.5 Å)

1995: Articulation of prospects of obtaining atomic resolution protein structures *without* crystals

2008: First near-atomic resolution icosahedral viral structures (3.9 Å)

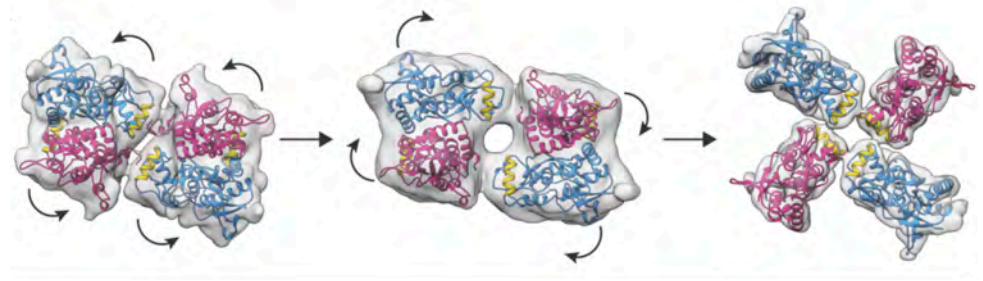
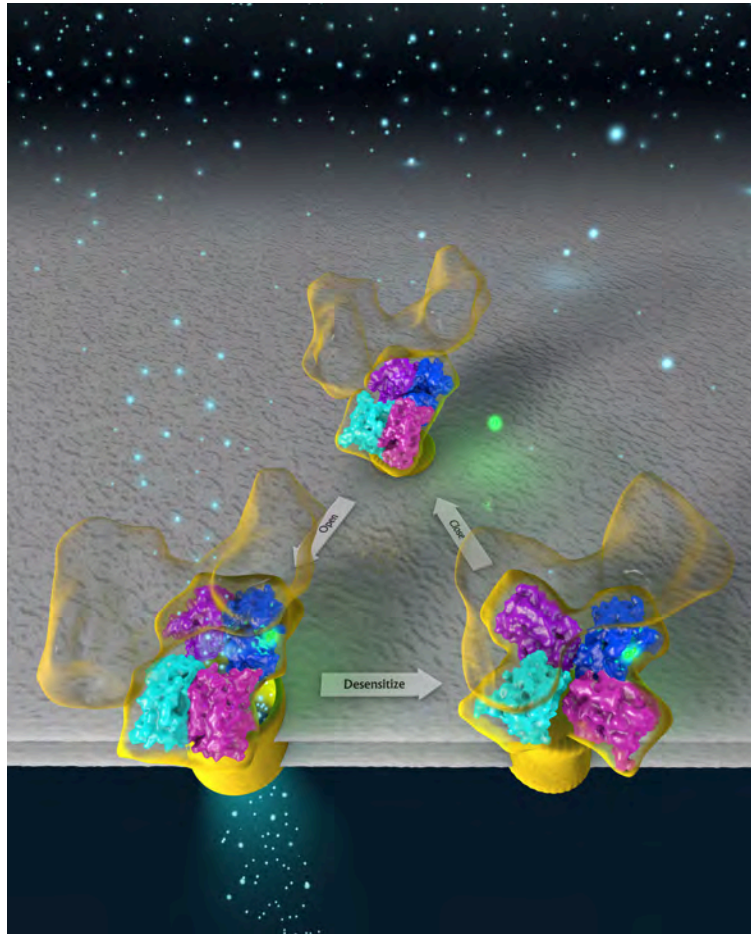
2013: First near-atomic resolution membrane protein structure (3.4 Å)

2014: Structure of a dynamic metabolic enzyme implicated in cancer (3.0 Å)

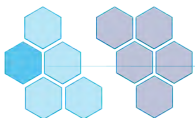




Glutamate receptor gating cycle

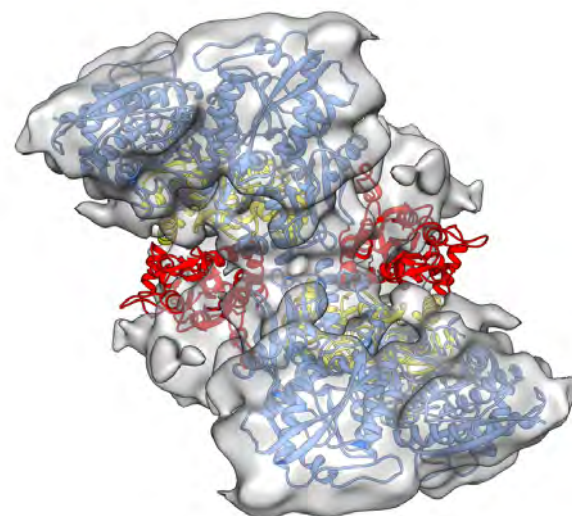
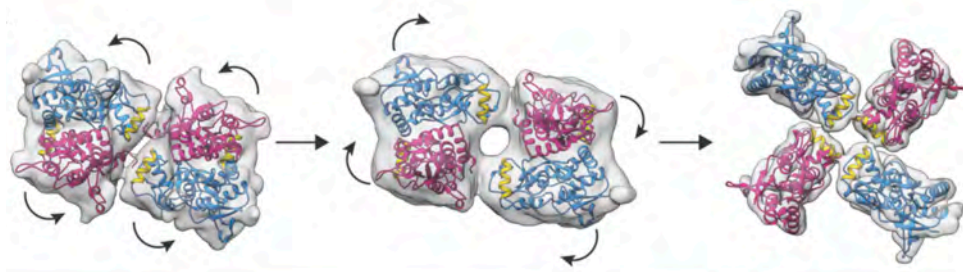
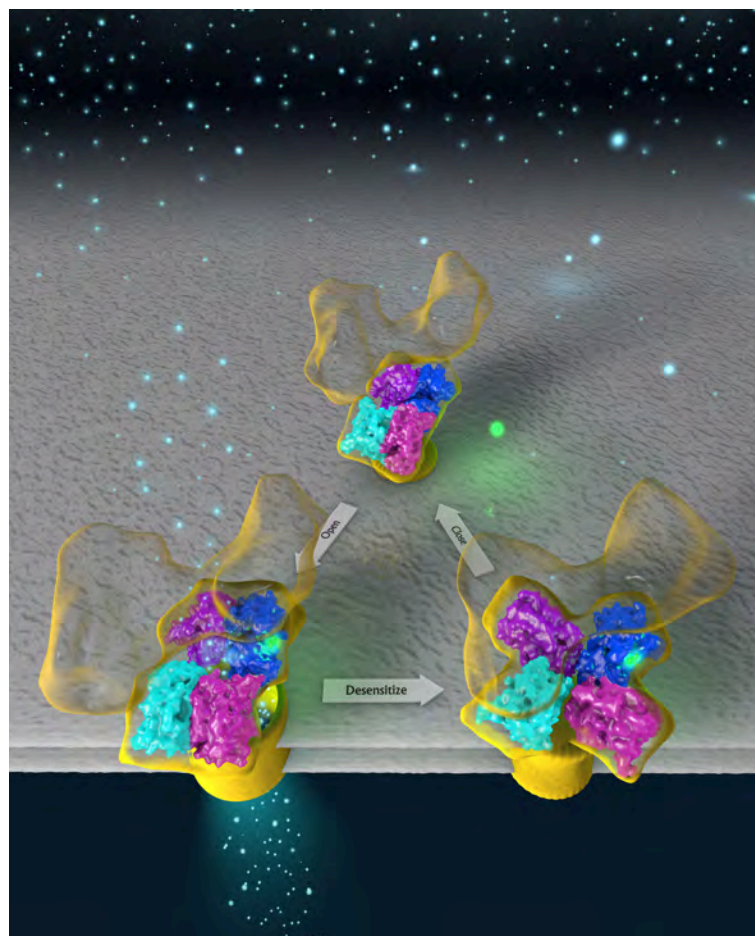


Meyerson et al *Nature* (2014)

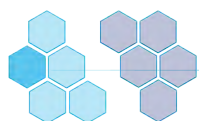




Glutamate receptor gating cycle

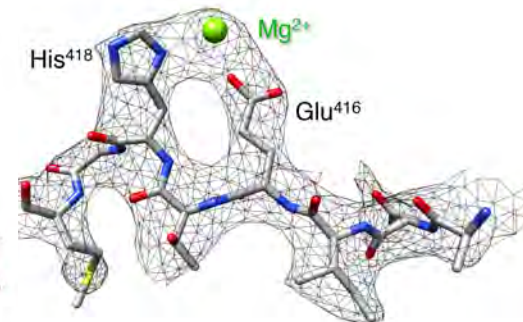
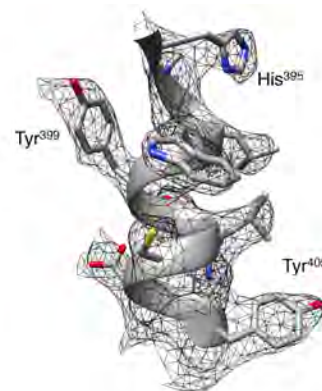
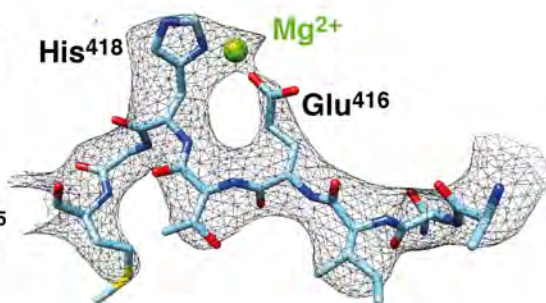
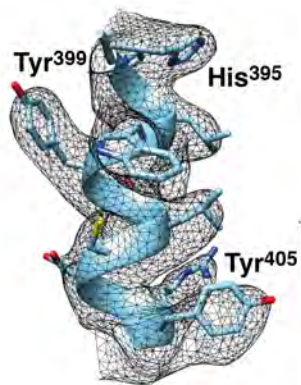
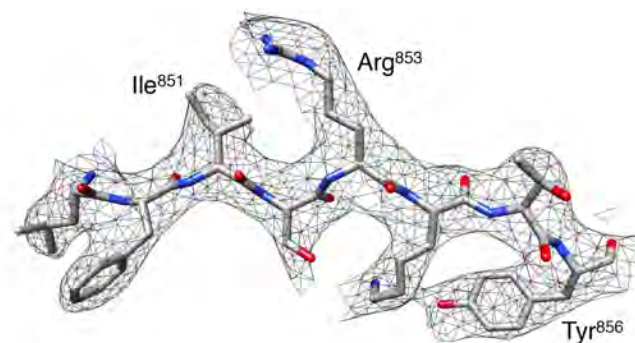
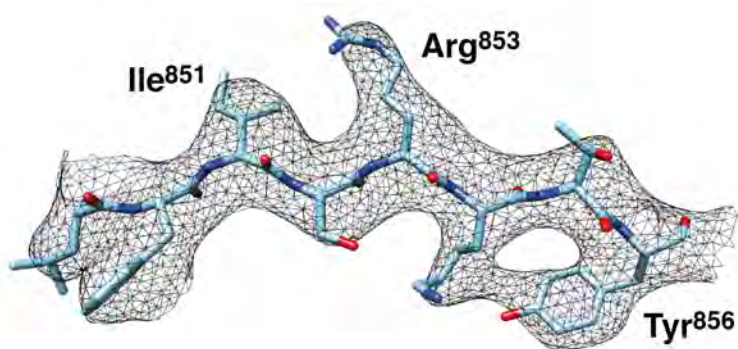


Precise knowledge of protein movements will enable design of drugs that trap distinct functional states





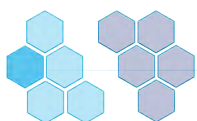
Comparison between cryo-EM and X-ray maps of β -galactosidase



cryo-EM
3.2 Å

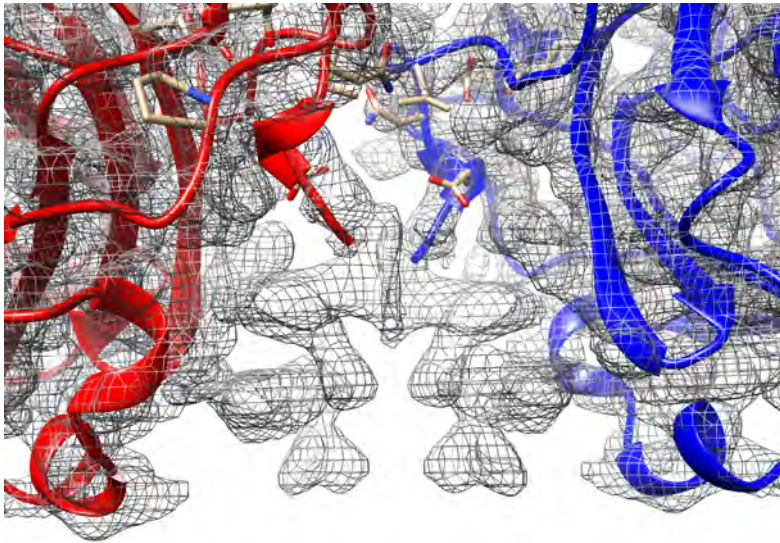
X-ray
3.0 Å

Bartesaghi et al *Proc. Natl. Acad. Sci. USA* (2014)

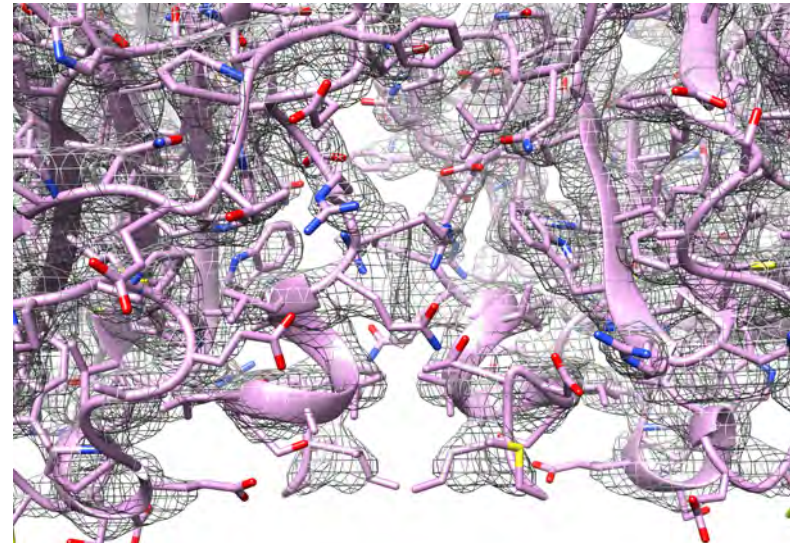




Cryo-EM structure of β -galactosidase at 3.2 Å resolution

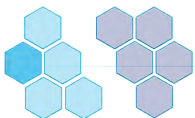


Fitted with X-ray
coordinates



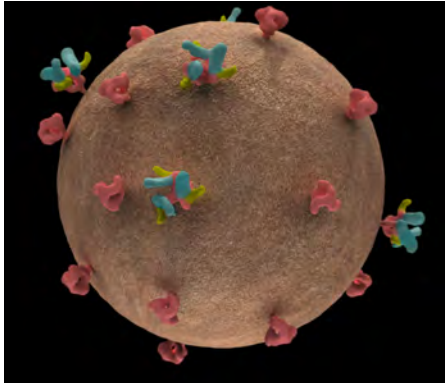
Fitted with cryo-EM
coordinates

Cryo-EM can provide atomic resolution structures of full-length proteins under native conditions

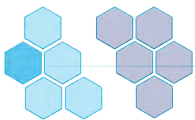




Understanding HIV entry: A multifaceted challenge

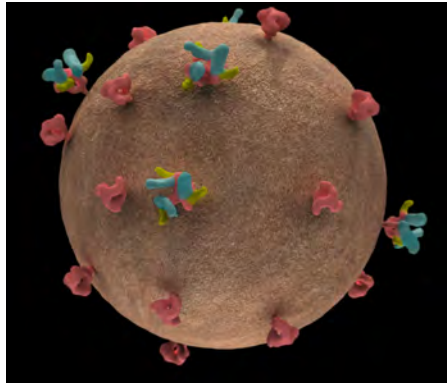


Antibody
neutralization

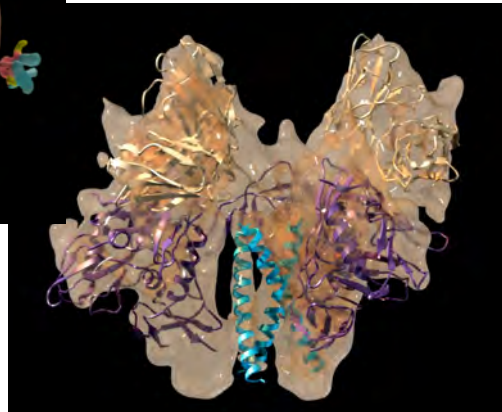




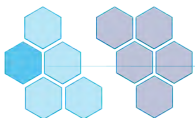
Understanding HIV entry: A multifaceted challenge



Antibody
neutralization

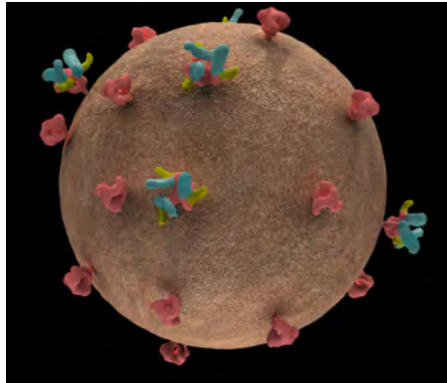


3D structure of
trimeric Env

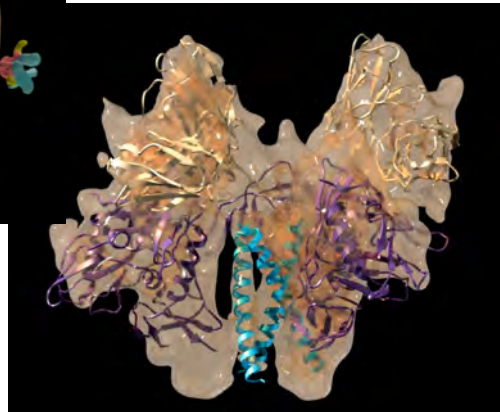




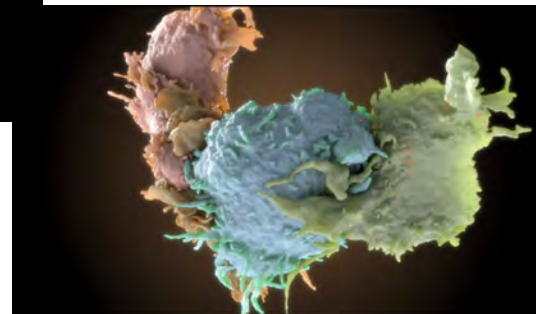
Understanding HIV entry: A multifaceted challenge



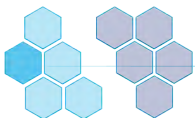
Antibody
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3D structure of
trimeric Env

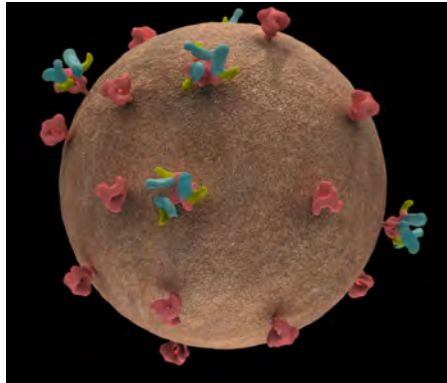


Cell-cell
transmission

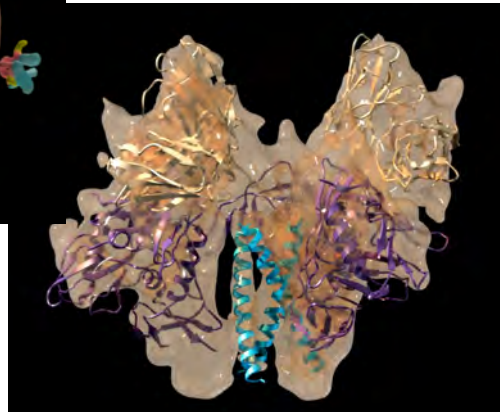




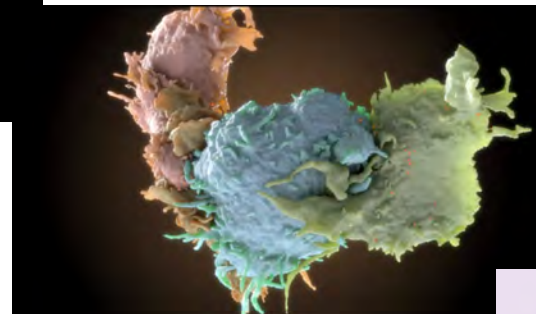
Understanding HIV entry: A multifaceted challenge



Antibody
neutralization

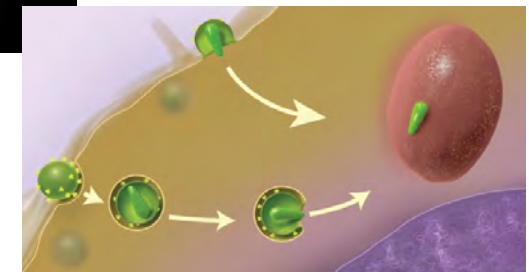


3D structure of
trimeric Env

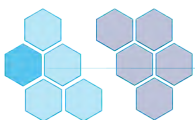


Cell-cell
transmission

**A complete understanding of the problem
requires integration of information across
cellular and molecular scales**

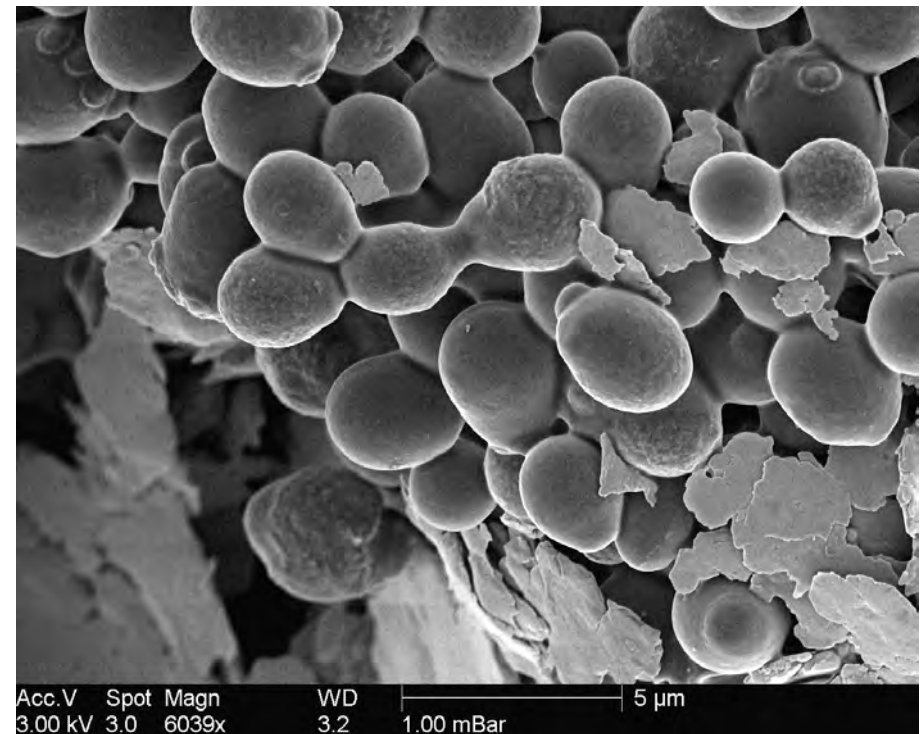
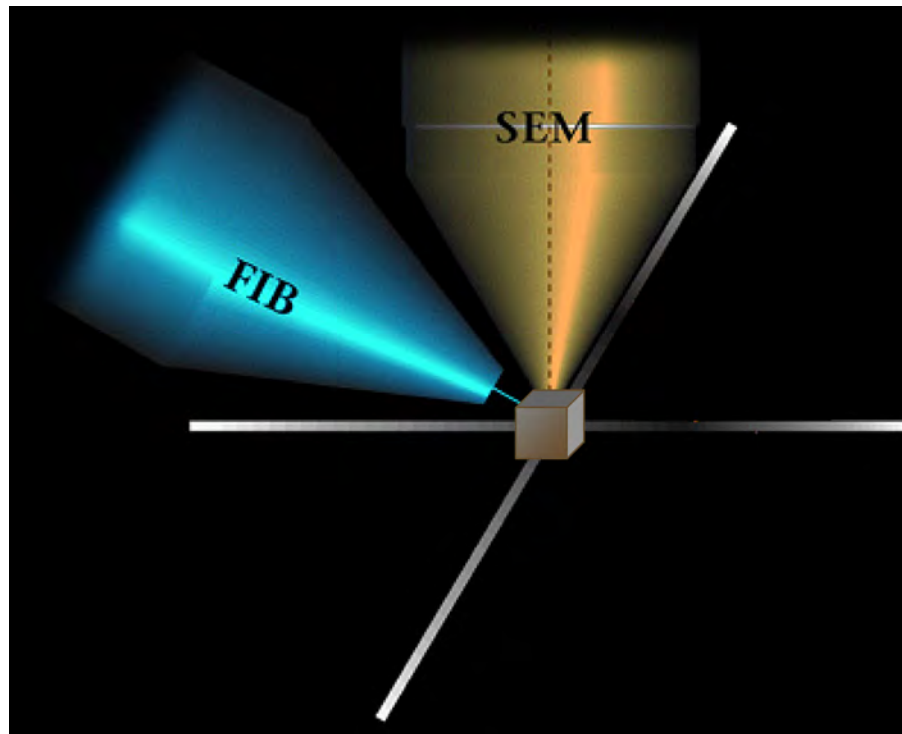


Pathway to the
nucleus

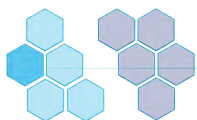




Focused ion beam Scanning Electron Microscopy for rapid 3D imaging of cells and tissue

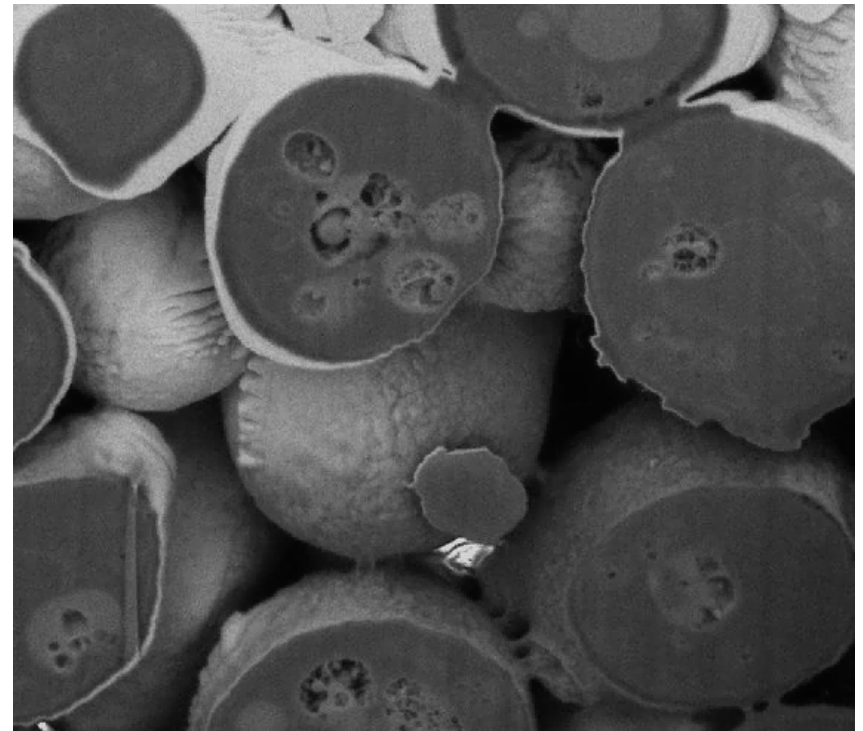
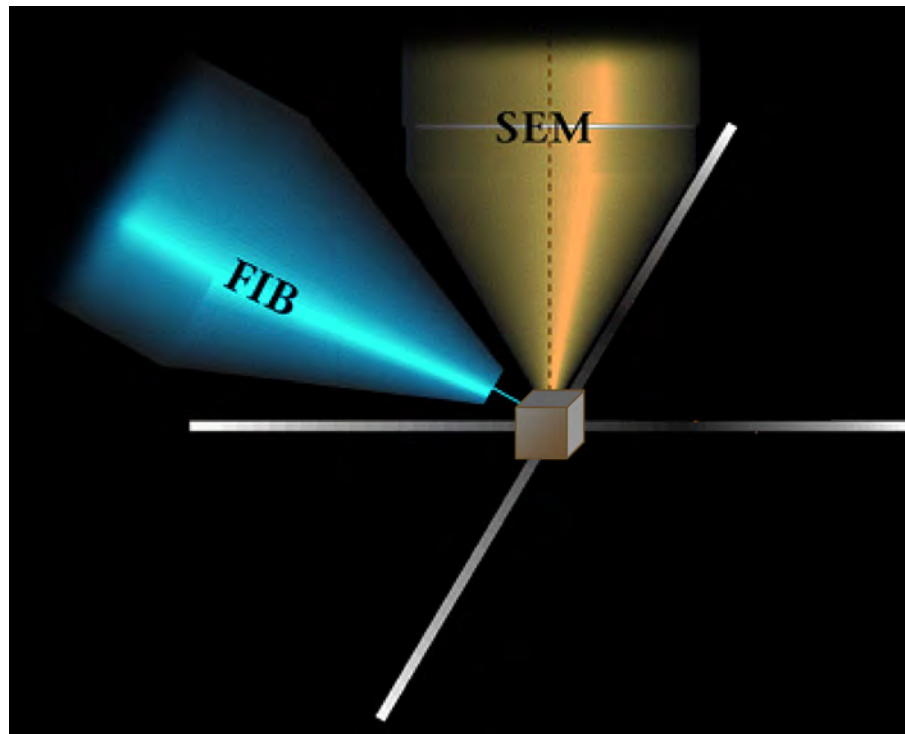


Iteration of slicing and imaging

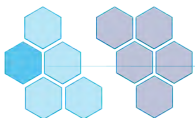




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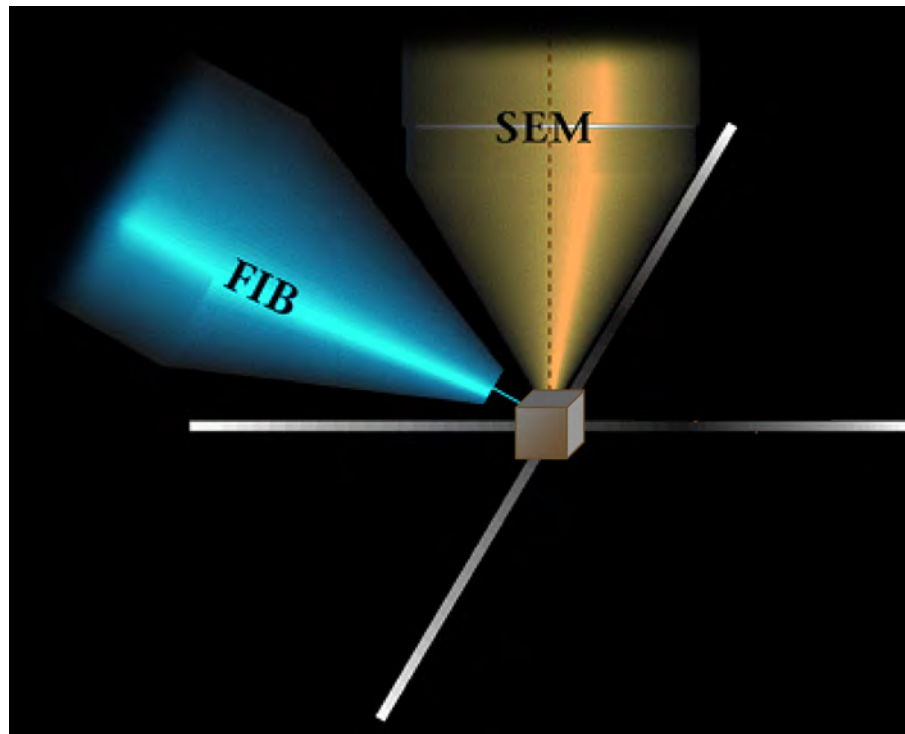


Iteration of slicing and imaging

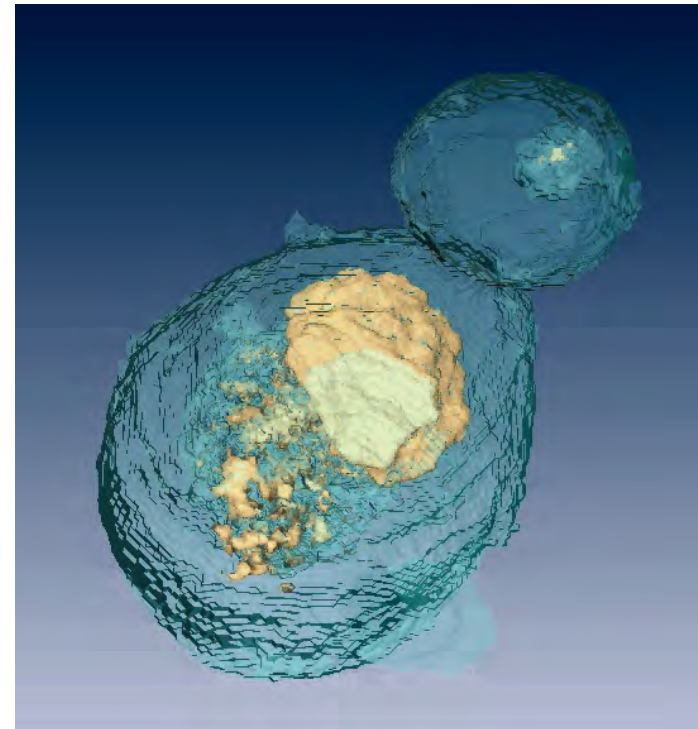




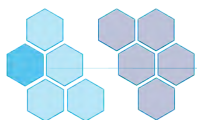
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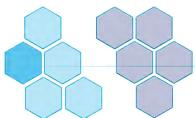
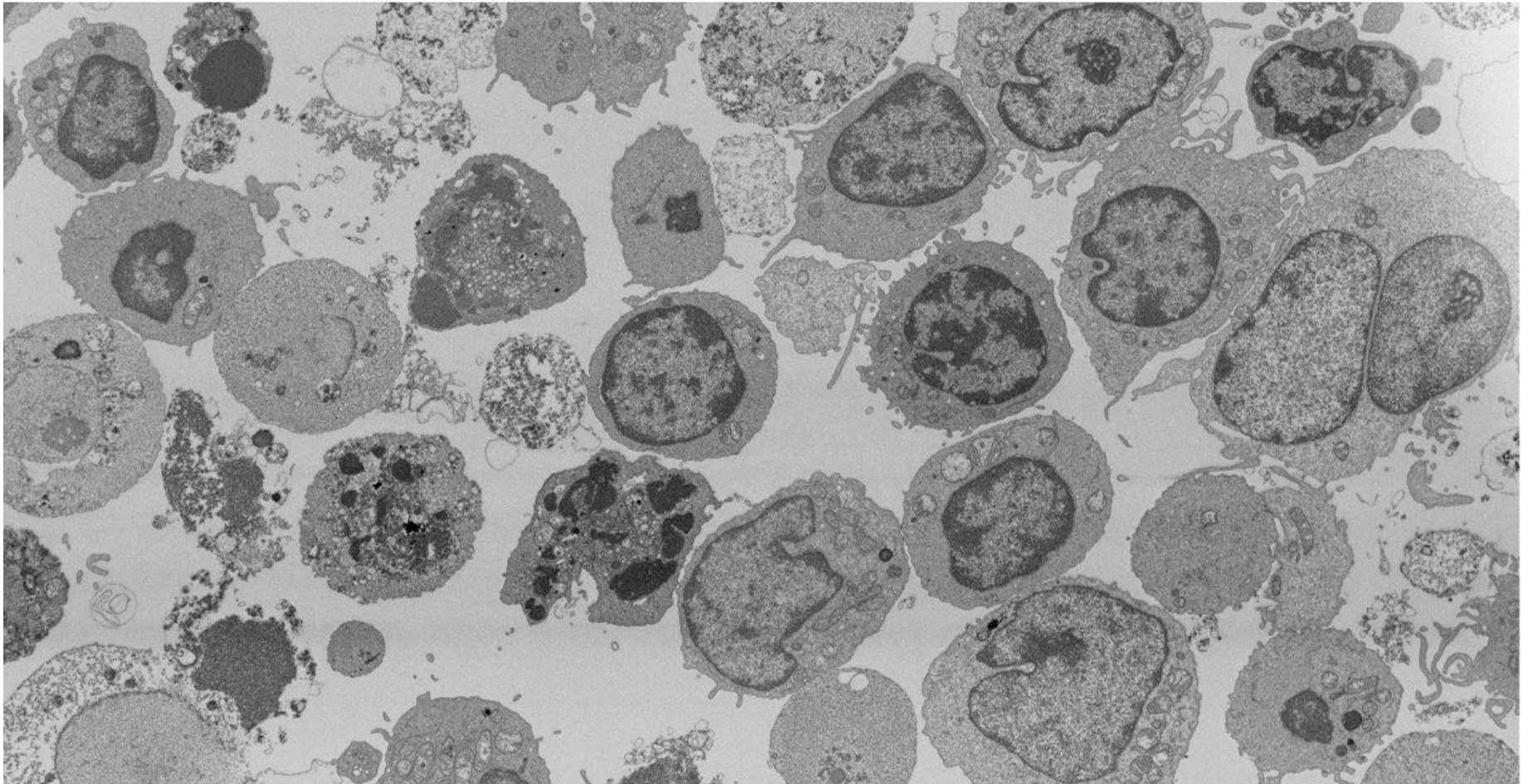


Heymann et al (2006)



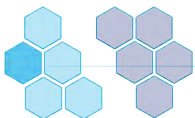
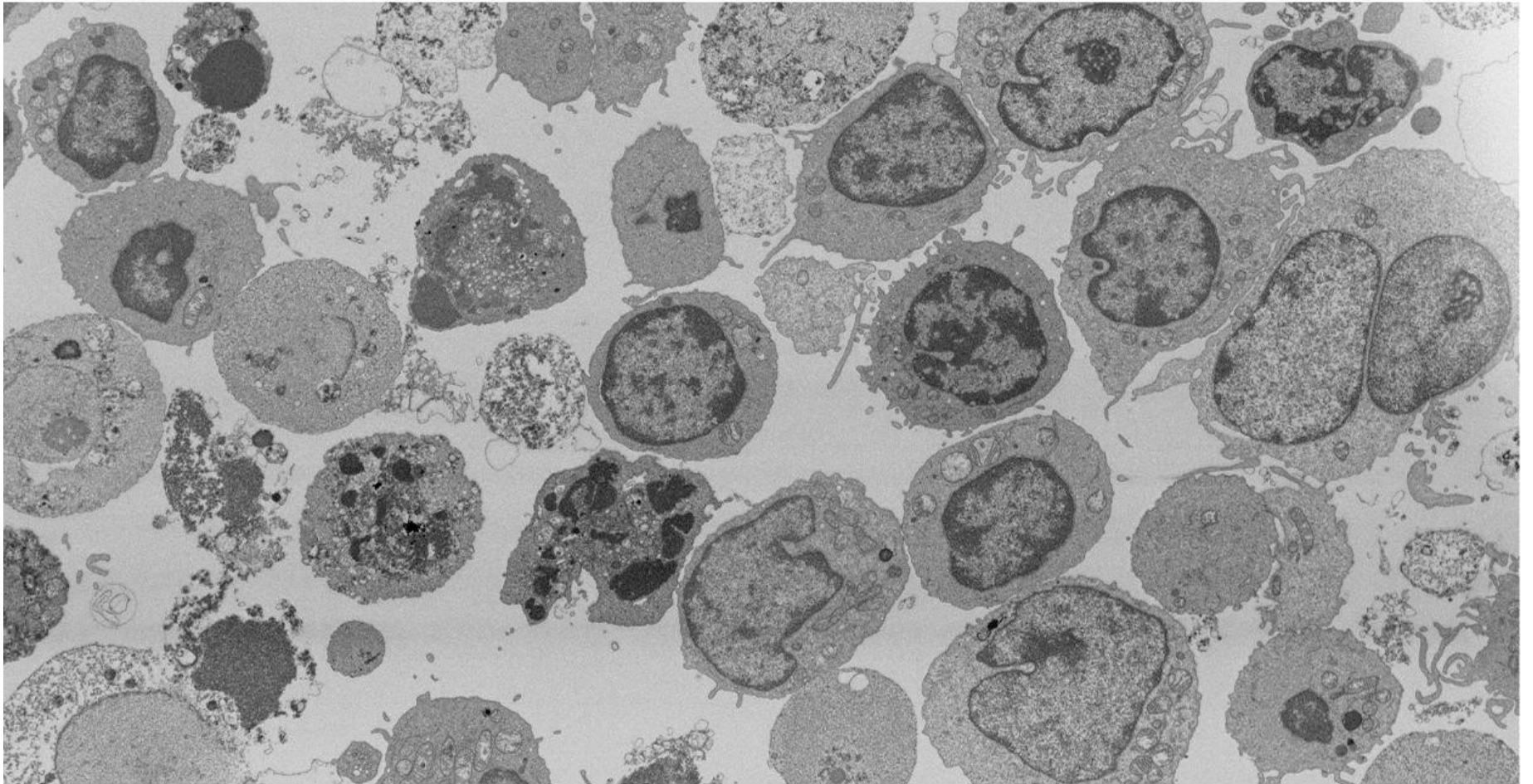


A journey into T-cell synapses



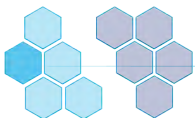
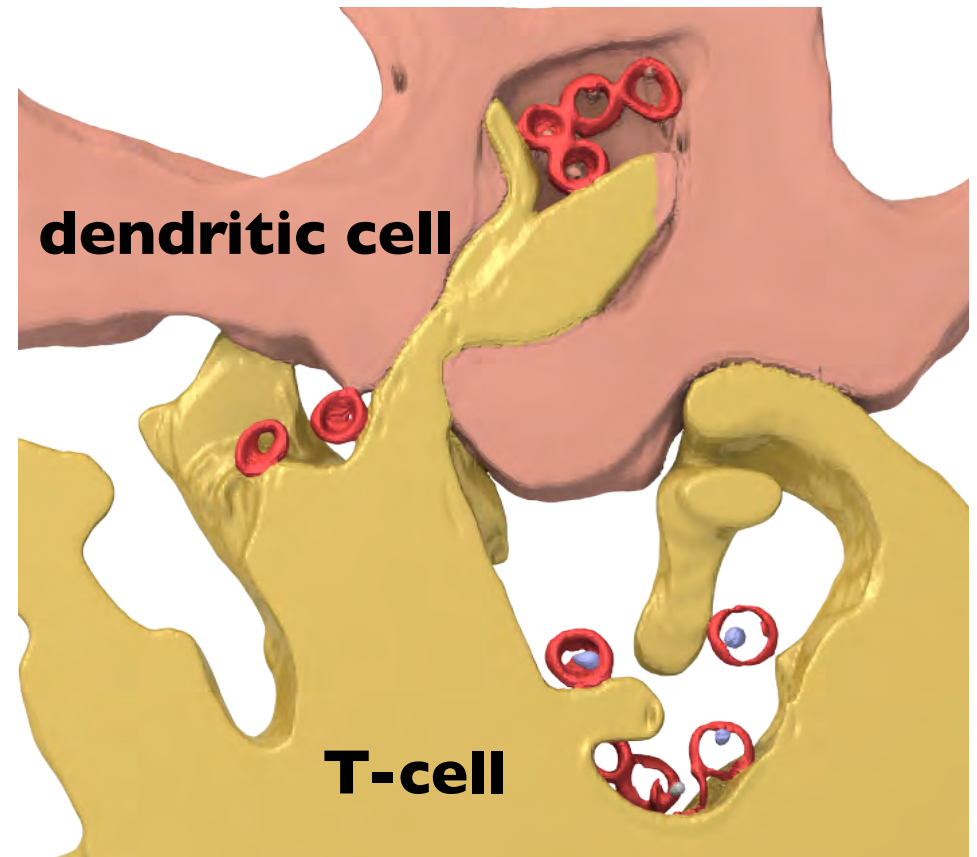
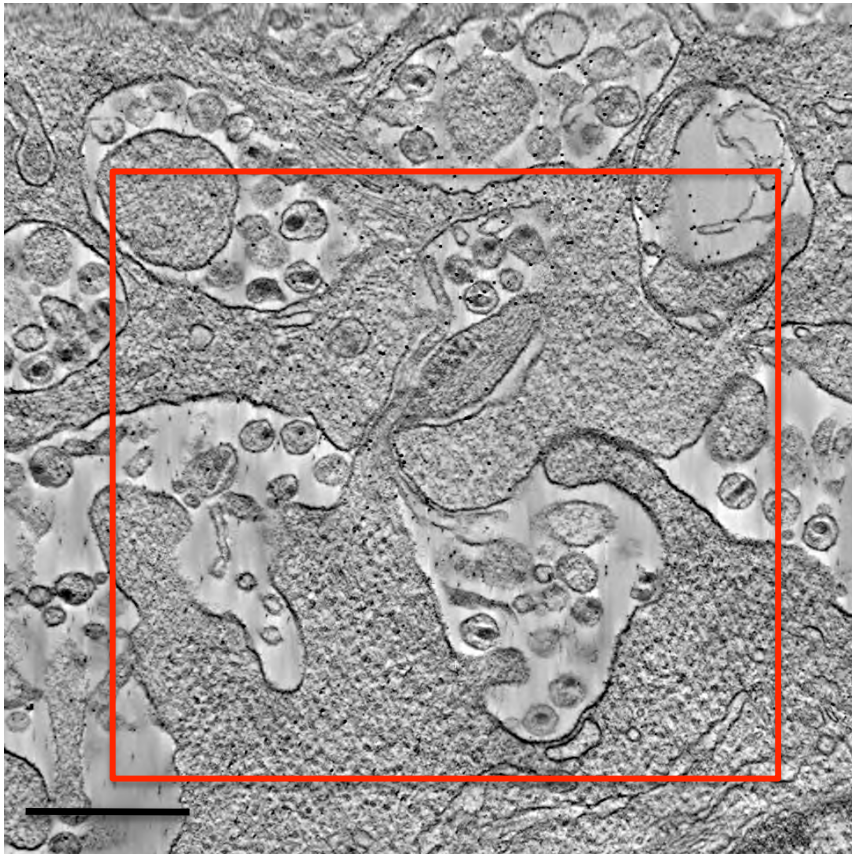


A journey into T-cell synapses



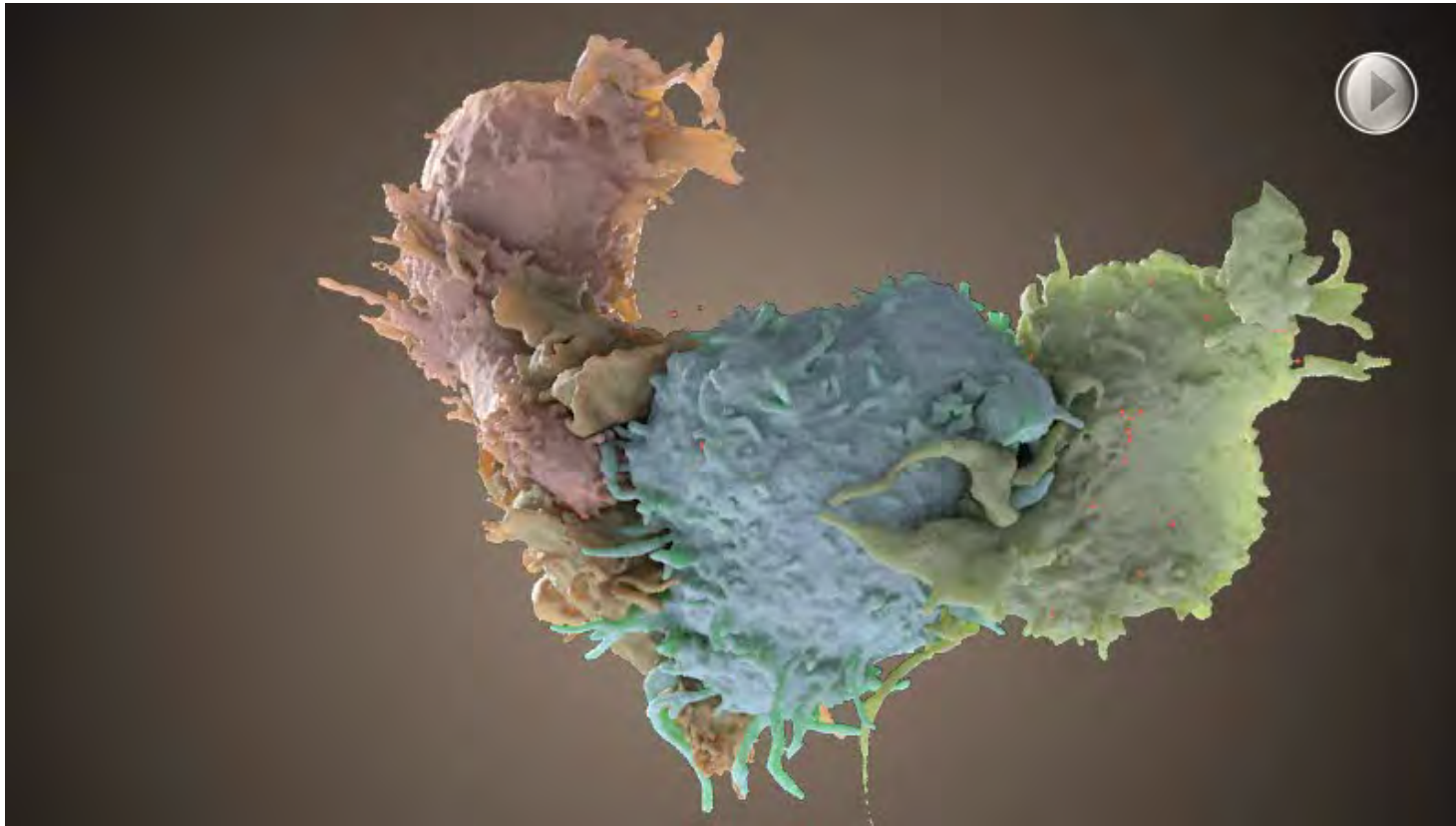


Intimate contact at the cell-cell interface

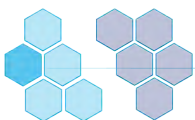




Synapses between primary T-cells

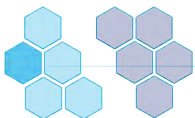
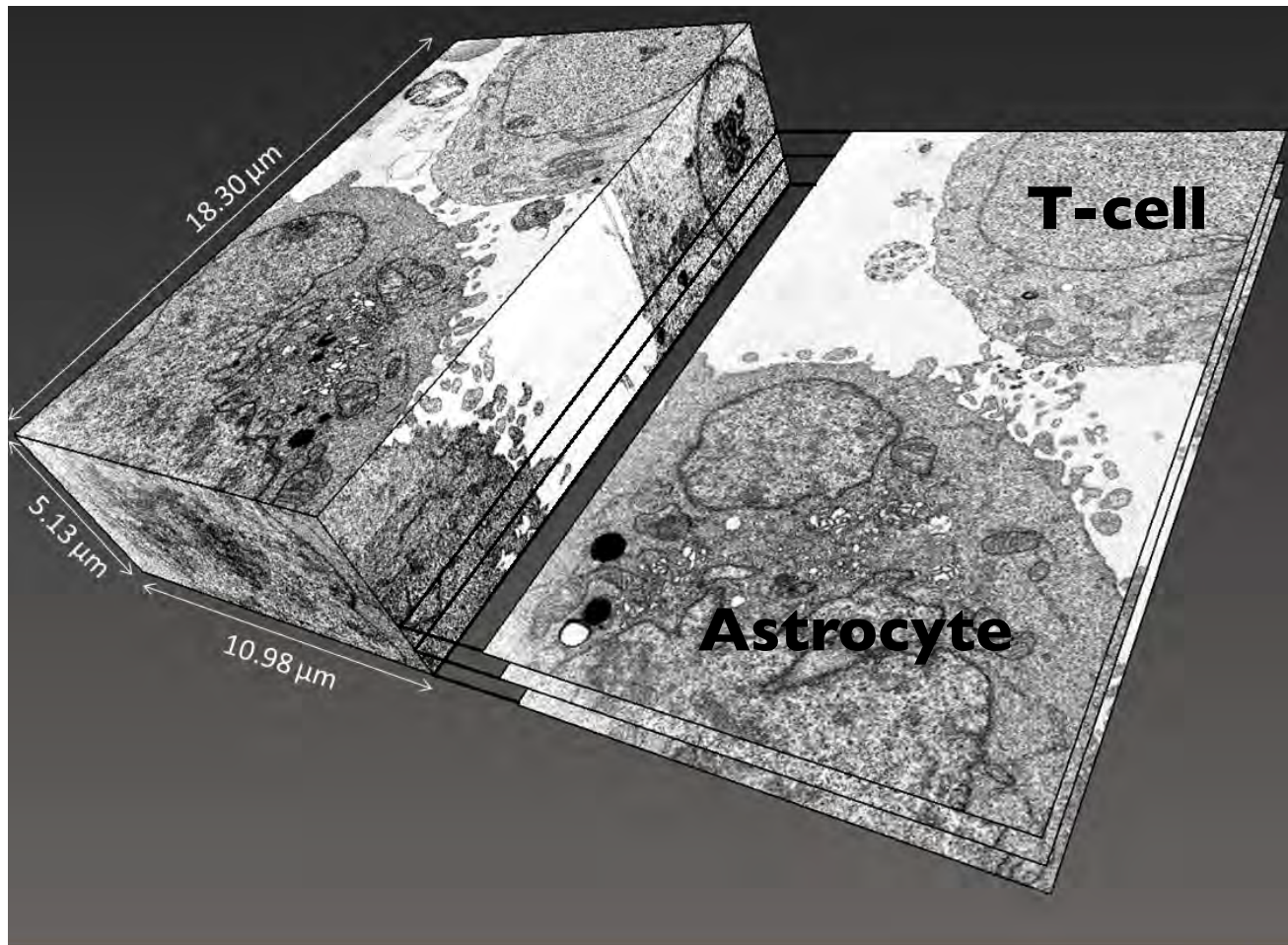


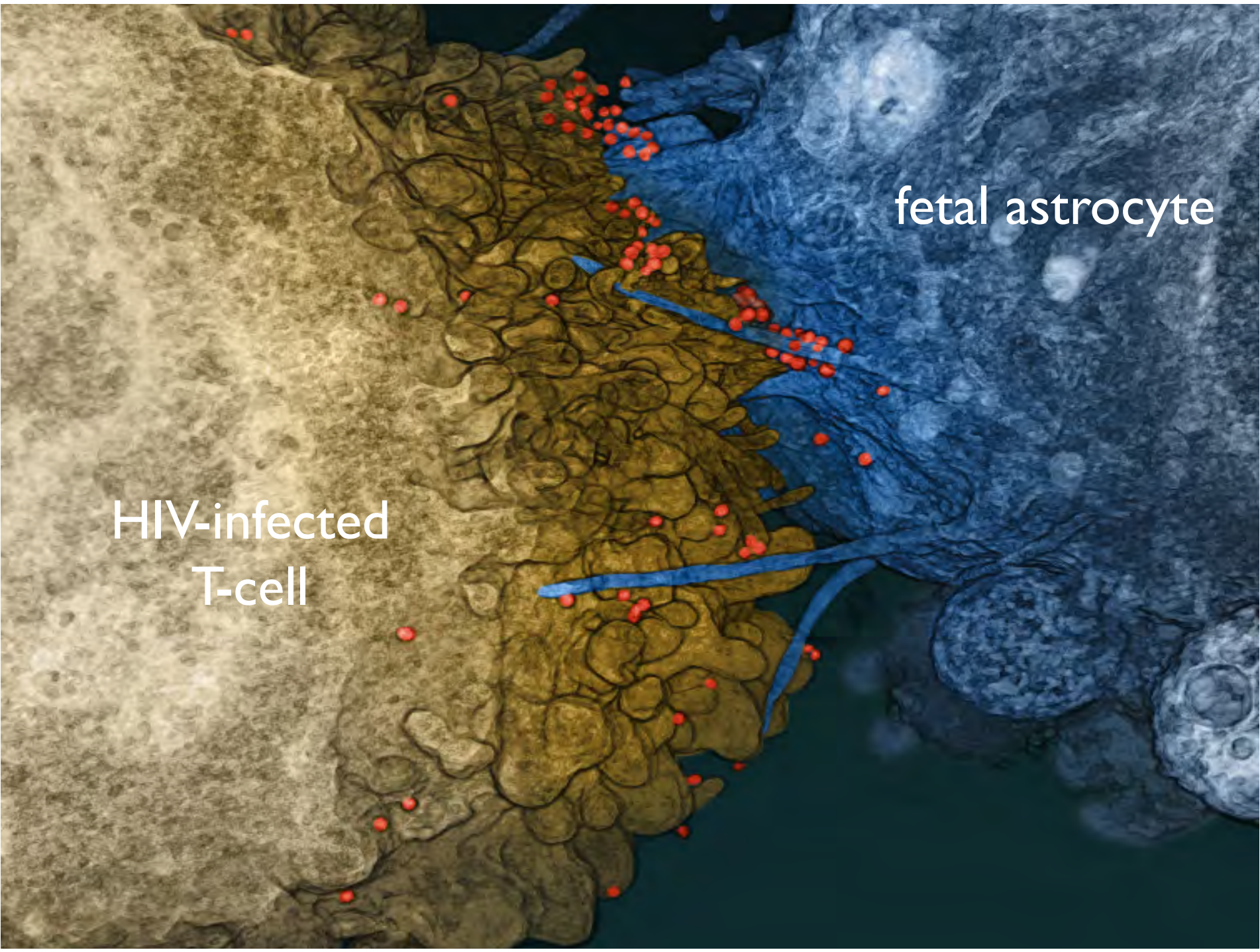
Do et al (2014)





HIV transfer to fetal astrocytes



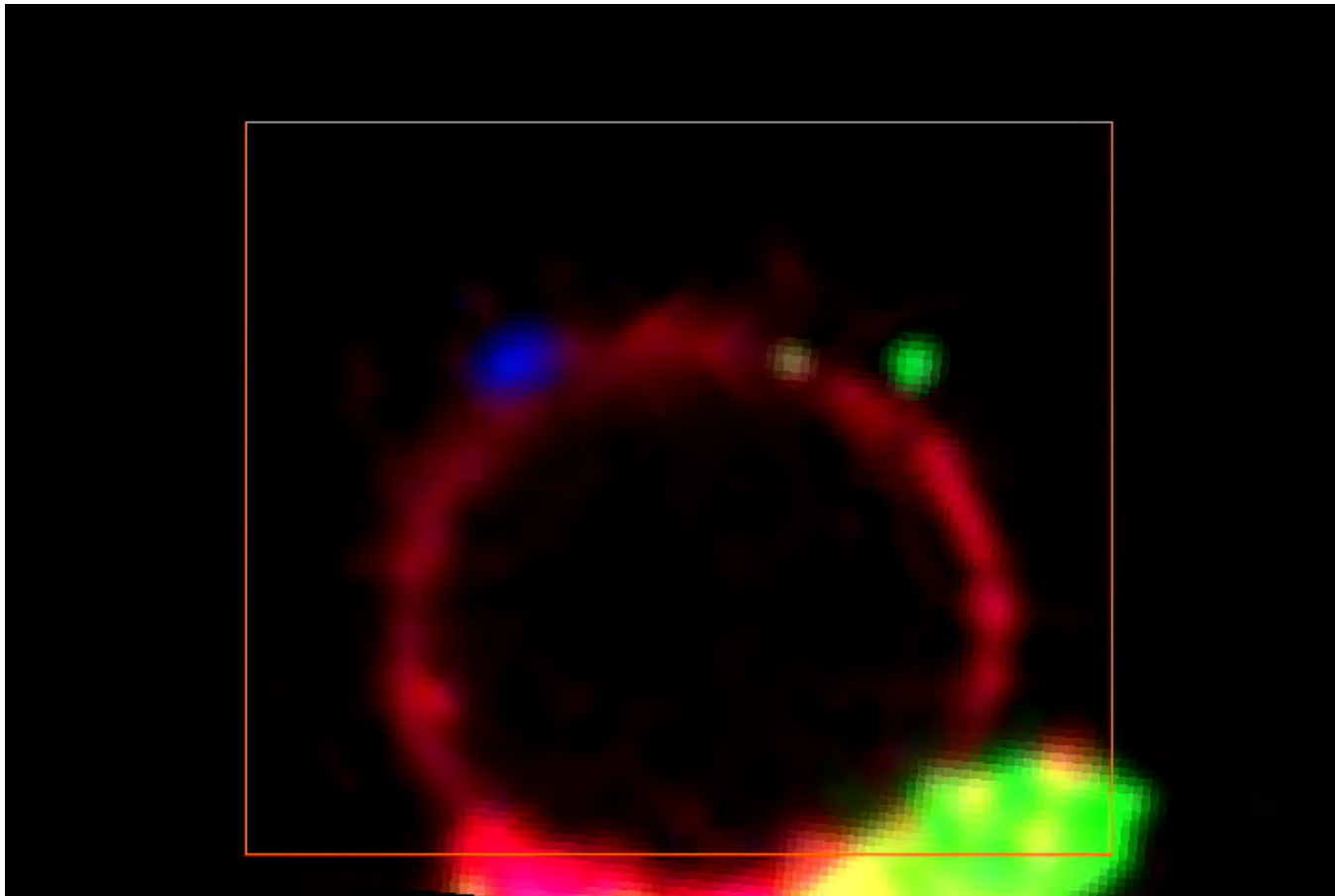


HIV-infected
T-cell

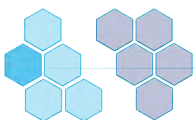
fetal astrocyte



Correlative live confocal and ion-abrasion SEM imaging: A cell biologist's dream

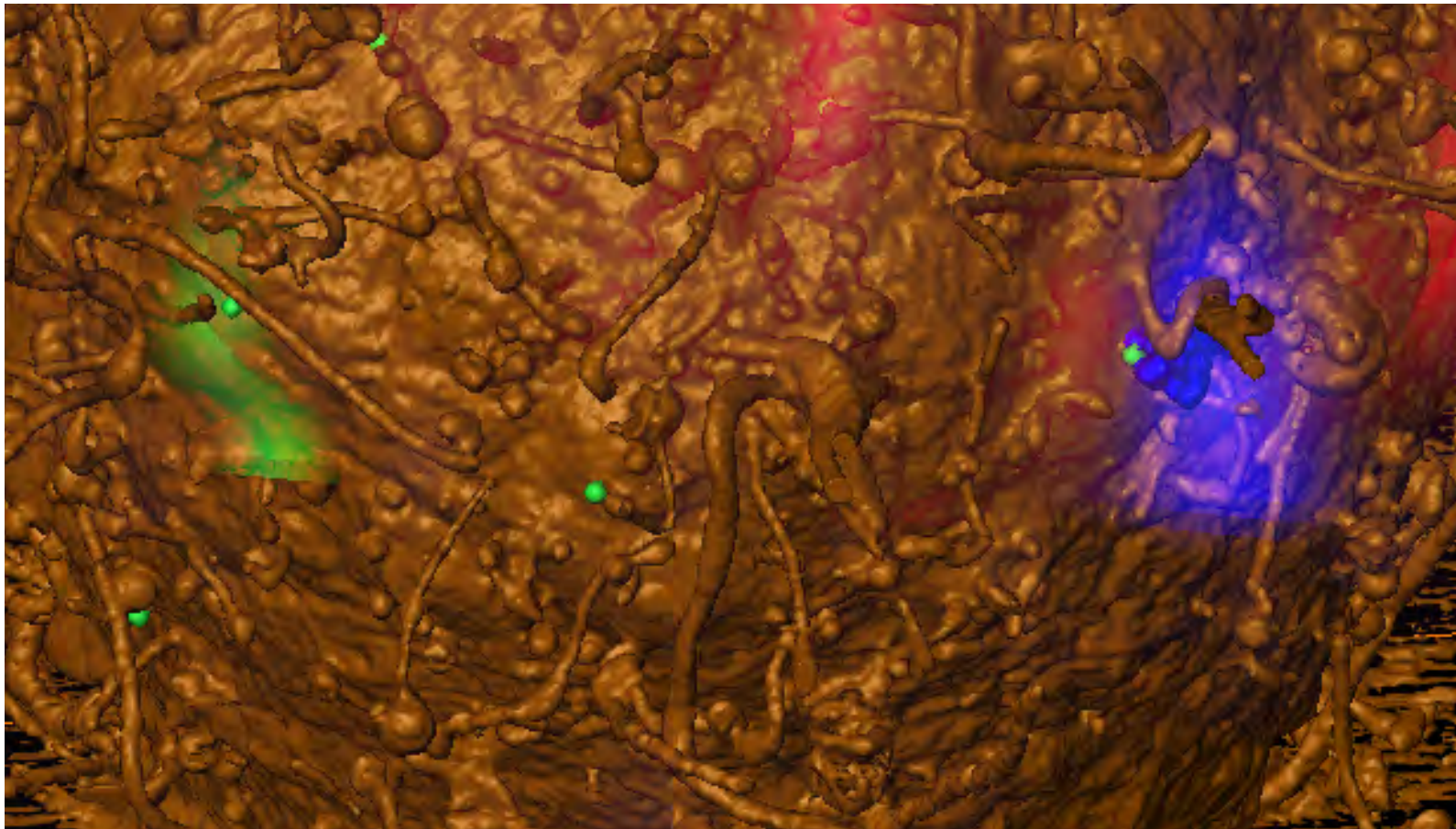


3D image of entire T-cell

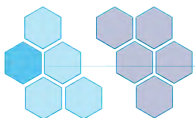




Correlative live confocal and ion-abrasion SEM imaging: A cell biologist's dream

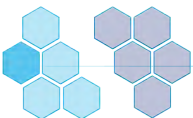
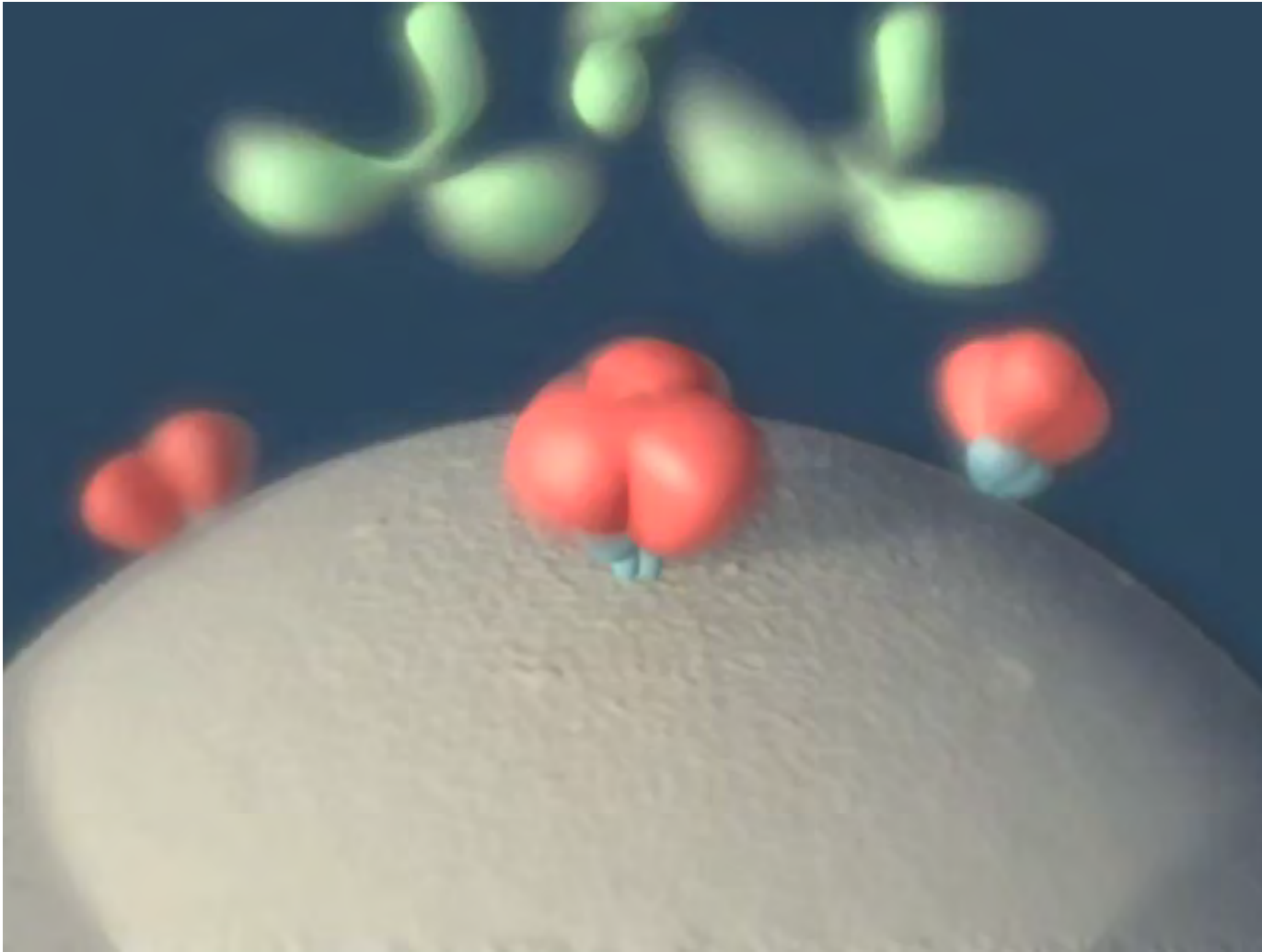


Narayan et al J. Struct. Biol. (2014)



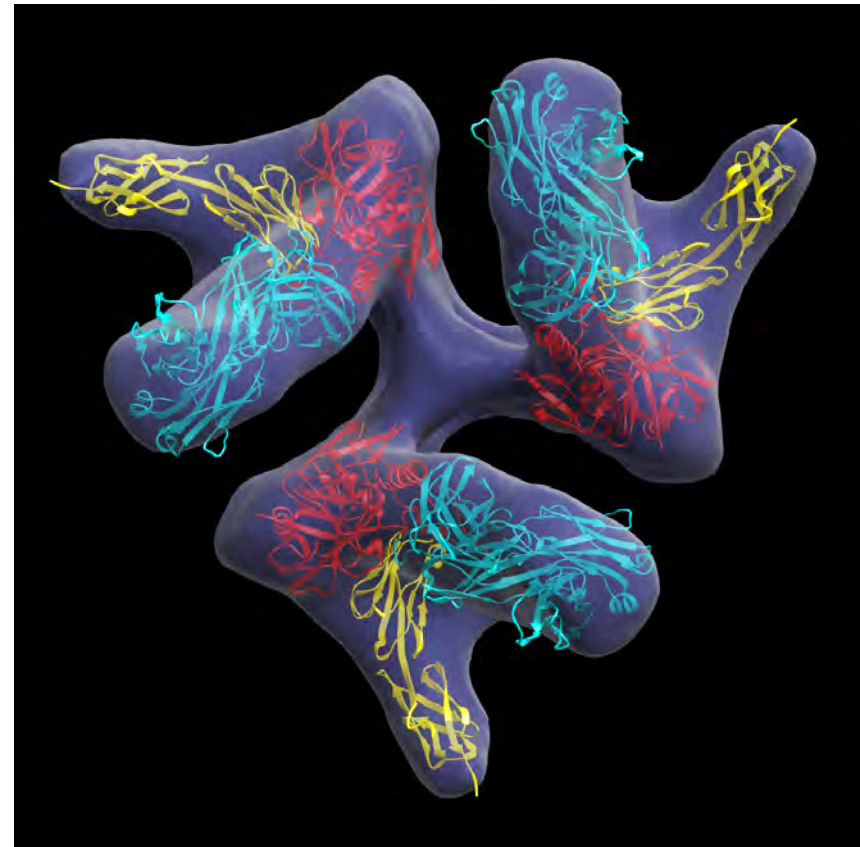
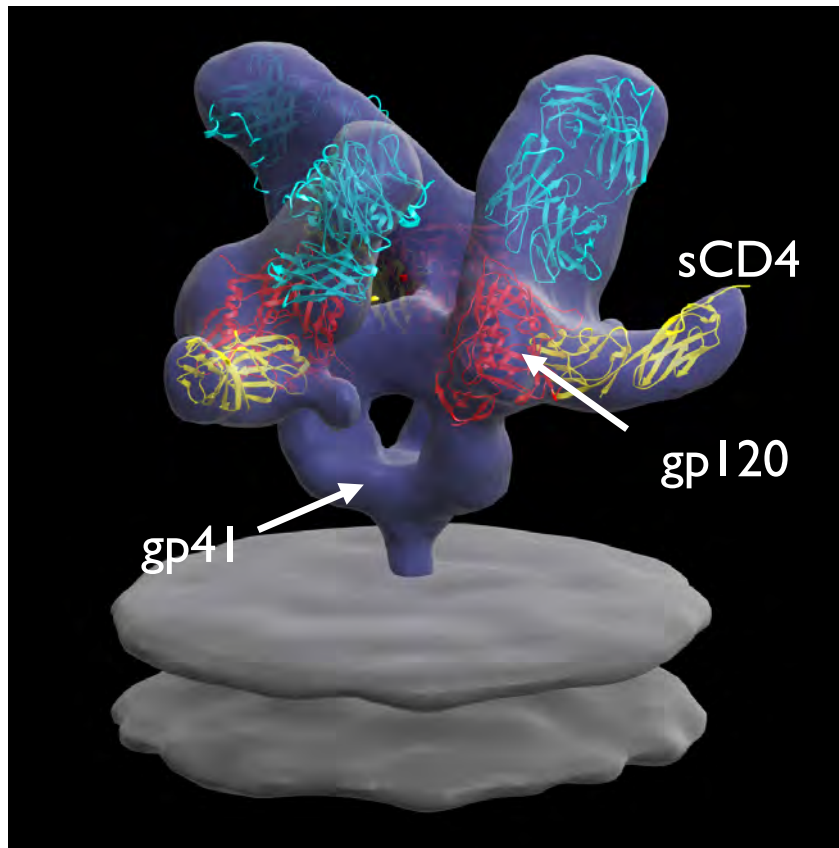


The dynamic HIV spike

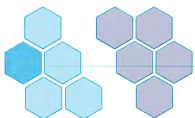




Molecular architecture of trimeric HIV envelope glycoproteins

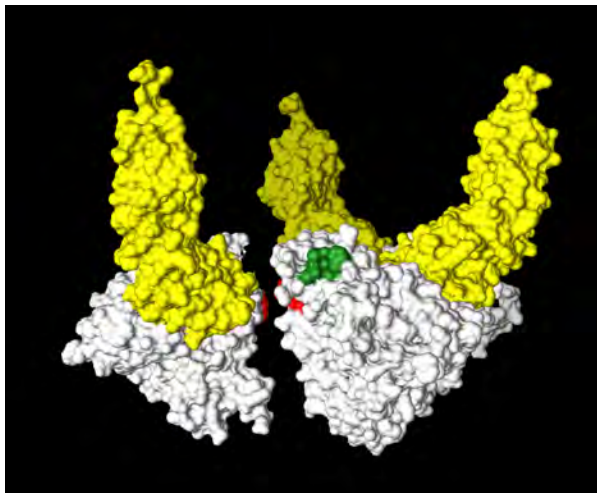


Subtomogram averages at ~ 20 Å resolution

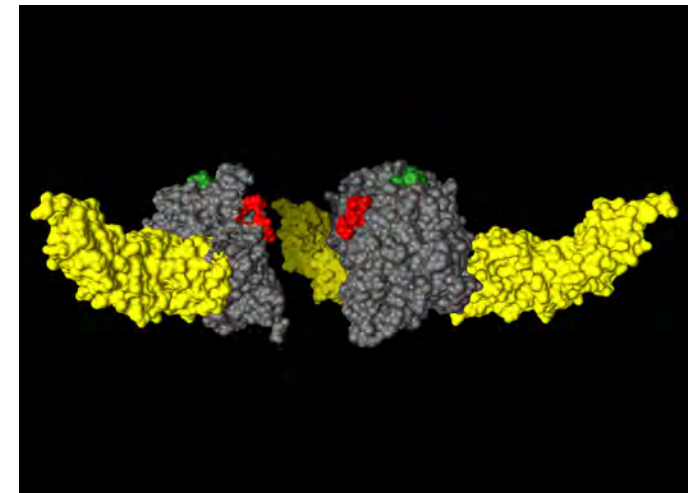




Catching HIV in the act with electron tomography

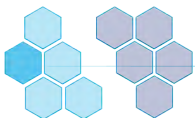


closed



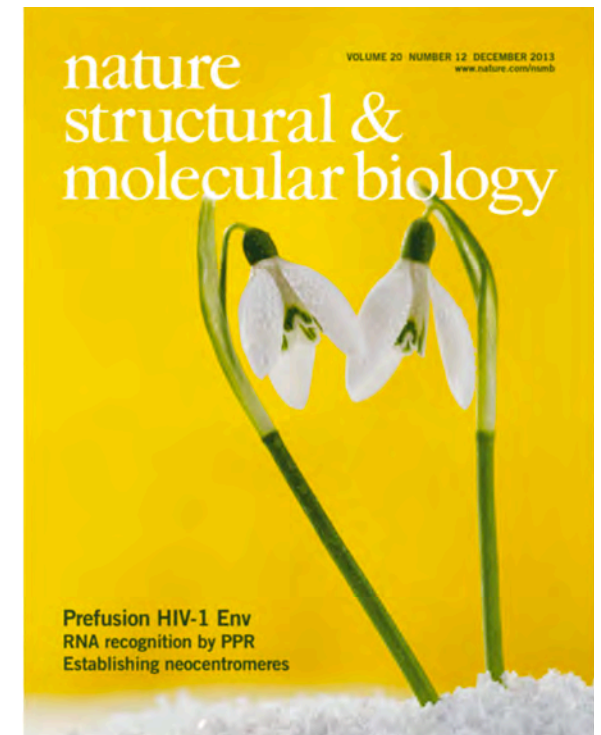
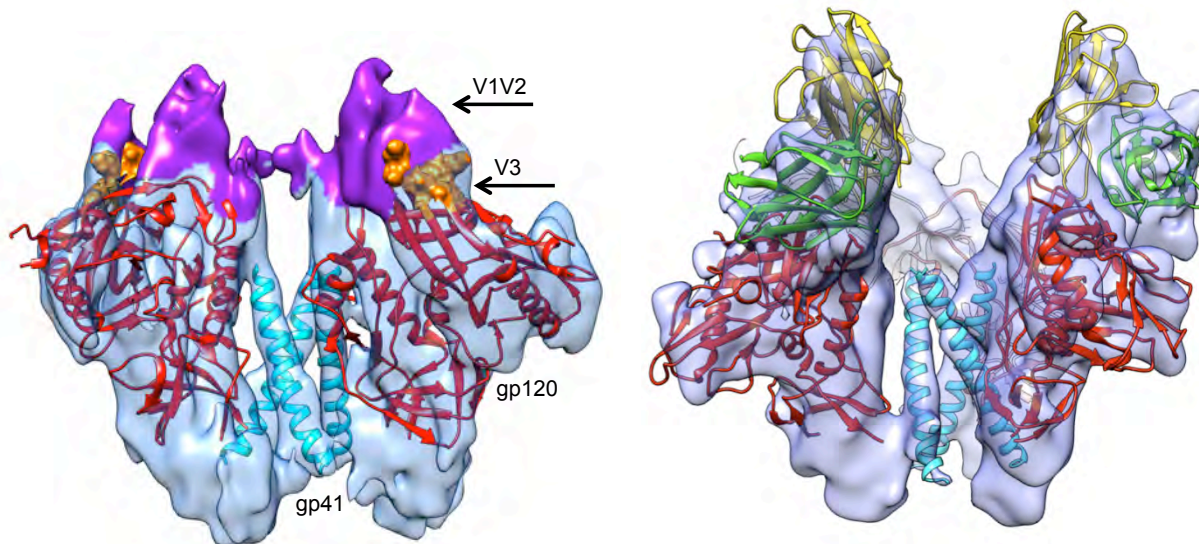
open

Liu et al, *Nature* (2008)
White et al *PLoS Path.* (2010)
Tran et al *PLoS Path.* (2012)
Meyerson et al *PNAS* (2013)

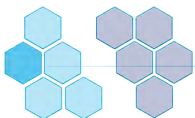




Structures of soluble HIV-1 Env immunogens at ~ 6Å - 9Å resolution



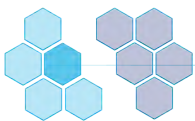
Bartesaghi et al (2013)





Why FNL?

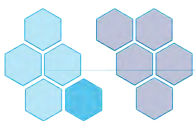
- c-CRADA mechanism for facile collaborations with industrial and academic collaborators
- Strong infrastructure can be established at FNL for collaborations requiring support for pre-microscopy (biochemistry) and post-microscopy (computing) applications
- CCR/NCI cryo-EM program already has footprint at ATRF
- Proximity to many leading institutions along East Coast with strong structural biology programs





Scope

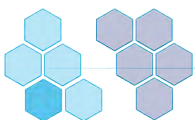
- Similarities and differences with DOE national laboratories that support high resolution electron microscopy
- National laboratory versus local academic user facilities
- Private sector and NIH-wide participation
- Synergy between components that provide user access to existing technologies versus those that develop breakthrough technologies
- Budget considerations





Leadership

- Set clear long-term vision for laboratory
- Important to maintain both technology development and routine user access components of laboratory
- Nucleation of highly motivated multi-disciplinary teams that can identify and tackle difficult challenges
- Effective strategies to stay at forefront of new developments in structural and cell biology
- Opportunity to establish internationally unique center





Training

- Mechanisms to host long-term and short-term visits from extramural researchers
- Core team of specialists to provide support in all aspects of structural investigation from biochemistry to computation
- Resident scholar program
- Strong training partnerships with neighboring institutions
- Peer review and competitive award by extramural study section panel

